PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference E30109PCT	**FOR FURTHER ACTION**	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
International application No. PCT/EP 03/02245	International filing date *(day/month/year)* 05.03.2003	Priority date *(day/month/year)* 05.03.2002
International Patent Classification (IPC) or both national classification and IPC C12Q1/68		
Applicant EPIGENOMICS AG et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 8 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 4 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the opinion
   - II ☐ Priority
   - III ☒ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☐ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
17.09.2003	08.04.2004
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized Officer Stachowiak, O Telephone No. +49 89 2399-7219

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/EP 03/02245

I. Basis of the report

1. With regard to the **elements** of the international application *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17))*:

Description, Pages

1-47 as originally filed

Claims, Numbers

1-15 received on 26.02.2004 with letter of 26.02.2004

Drawings, Sheets

1/7-7/7 as originally filed

Sequence listing part of the description, pages:

48-49, as originally filed

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

   These elements were available or furnished to this Authority in the following language: , which is:

   ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).

   ☐ the language of publication of the international application (under Rule 48.3(b)).

   ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

   ☒ contained in the international application in written form.

   ☒ filed together with the international application in computer readable form.

   ☐ furnished subsequently to this Authority in written form.

   ☐ furnished subsequently to this Authority in computer readable form.

   ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

   ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

   ☐ the description, pages:

   ☐ the claims, Nos.:

   ☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)).

   (Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:

   ☐ the entire international application,

   ☒ claims Nos. 1-14 (all partly)

   because:

   ☒ the said international application, or the said claims Nos. 1-14 (all partly) relate to the following subject matter which does not require an international preliminary examination (specify):

   see separate sheet

   ☐ the description, claims or drawings *(indicate particular elements below)* or said claims Nos. are so unclear that no meaningful opinion could be formed *(specify)*:

   ☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.

   ☐ no international search report has been established for the said claims Nos.

2. A meaningful international preliminary examination cannot be carried out due to the failure of the nucleotide and/or amino acid sequence listing to comply with the standard provided for in Annex C of the Administrative Instructions:

   ☐ the written form has not been furnished or does not comply with the Standard.

   ☐ the computer readable form has not been furnished or does not comply with the Standard.

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-15
	No:	Claims	
Inventive step (IS)	Yes:	Claims	
	No:	Claims	1-15
Industrial applicability (IA)	Yes:	Claims	1-15
	No:	Claims	

2. Citations and explanations

see separate sheet

Re Item III

Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

3.1 For the assessment of present claims 1-14 on the question whether they are industrially applicable, no unified criteria exist in the PCT Contracting States. Claims 1-14 relate to methods which comprise the step of 'obtaining' or 'retrieving' a bodily fluid sample from an individual (step a), a step which may render such a claim not allowable under certain national and/or regional legislations. The patentability can also be dependent upon the formulation of the claims. The EPO, for example, does not recognize as industrially applicable the subject-matter of claims directed to the treatment of the human or animal body by surgery or therapy, and diagnostic methods practised on the human or animal body.

Re Item V

Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

5.1 The following documents (D) are referred to in this communication; the numbering will be adhered to in the rest of the procedure:

D1: USADEL HENNING ET AL: 'Quantitative adenomatous polyposis coli promoter methylation analysis in tumor tissue, serum, and plasma DNA of patients with lung cancer.' CANCER RESEARCH, vol. 62, no. 2, 15 January 2002 (2002-01-15), pages 371-375, XP001080048 January 15, 2002 ISSN: 0008-5472

D2: SHAPIRO B ET AL: 'DETERMINATION OF CIRCULATING DNA LEVELS IN PATIENTS WITH BENIGN OR MALIGNANT GASTRO INTESTINAL DISEASE' CANCER (PHILADELPHIA), vol. 51, no. 11, 1983, pages 2116-2120, XP001083835 ISSN: 0008-543X

D3: LEON S A ET AL: 'FREE DNA IN THE SERUM OF CANCER PATIENTS AND THE EFFECT OF THERAPY' CANCER RESEARCH, vol. 37, no. 3, 1977, pages 646-650, XP001083834 ISSN: 0008-5472

D4: GIACONA M B ET AL: 'CELL-FREE DNA IN HUMAN BLOOD PLASMA: LENGTH MEASUREMENTS IN PATIENTS WITH PANCREATIC CANCER AND HEALTHY CONTROLS' PANCREAS, RAVEN PRESS, NEW YORK,

NY, US, vol. 17, no. 1, July 1998 (1998-07), pages 89-97, XP000992791 ISSN: 0885-3177

D5: BOTEZATU IRINA ET AL: 'Genetic analysis of DNA excreted in urine: A new approach for detecting specific genomic DNA sequences from cells dying in an organism.' CLINICAL CHEMISTRY, vol. 46, no. 8 Part 1, August 2000 (2000-08), pages 1078-1084, XP001080049 ISSN: 0009-9147

D6: US-B-6 214 556 (OLEK ALEXANDER ET AL) 10 April 2001 (2001-04-10)

INVENTIVE STEP:

5.2 With respect to claims 1-2:

Document D1 is related to a method for detecting the presence or absence of cancer (i.e., a 'diseased condition') in a tissue or organ of an individual comprising the steps of obtaining a bodily fluid sample and a cell sample, determining the amount of **methylated DNA** originating from that organ or tissue in the serum and cell sample, and determining the presence or absence of a diseased condition based on the methylation of DNA (APC promoter) originating from that organ or tissue (D1, abstract; page 372, first column, second paragraph - page 373, second column, last paragraph; Figures 1-3). As the method of claim 1 employs DNA methylation patterns rather than **DNA quantity itself** as an indicator for a pathological condition/disease, and claim 1 employs the **amount of free-floating DNA** as an indicator therefor, the latter claim is considered formally novel in the light of D1. The same applies with respect to the disclosures of D2-D5. The feature of claim 1 stating that the DNA 'originates from said tissue, cell type or organ' is consiederd to be disclosed by D1 as the latter document discloses methylation analysis of specific DNA regions which are indicative of a certain tissue, cell type or organ (cf. D1,

5.3 D1 is chosen as closest prior art because it serves the same general purpose as claim 1 and shares most of the features therewith. As stated above, the difference between claim 1 and D1 is that D1 employs methylation analysis of serum- and cell-derived samples, whereas claim 1 merely relates to the amount of DNA as an indicator of a diseases condition. The technical effect generated by that difference is that the method of claim 1 allows for an easy and convenient detection of a diseased condition. It is thus the problem of claim 1 to provide an alternative method for the detection of a diseased condition which is less cumbersome. The problem is solved by the method of claim 1.

5.4 However, D1 expressly discloses the fact that the serum of diseased patients i.e., of cancer patients) contains elevated levels of DNA (i.e., 4-40 times more than ususal, cf. D1, p. 371, col. 1, last para.). Furthermore, D1 also discloses tumor-specific alterations in the serum of patients (D1, p. 371, col. 1, last para - col. 2, first para.). Thus, taking the method of D1 as a starting point, it would have been obvious to the skilled person to apply the knowledge about the elevated serum and plasma DNA levels in cancer patients (cf. ibid.) to arrive at a method based on the measuremt of free DNA levels in a body fluid such as serum. Thus, the person skilled in the art would have combined the knowledge presented in D1 in order to arrive at the method of claim 1. Hence, claim 1 is considered obvious in the light of D1 taken alone. The same applies with respect to claim 2, because the skilled person would also routinley consider to compare the fraction of free floating DNA from a particular tissue to the total amount of free floating DNA.

5.5 With respect to claims 3-4:
A method comprising conditioning of the sample before detecting the amount of DNA is understood as any (bio-)chemical step which may be performed with the sample before DNA analysis. Thus, claims 3-4 are considered routine embodiments of a non inventive method, which do not encompass an inventive step.

5.6 With respect to claims 5-8:
A method in which the characteristic DNA methylation pattern is analyzed is disclosed in D1 (D1, abstract), as well as a method characterized in that the methylation pattern found is involved in the medical condition of interest (D1, abstract; Figure 2; page 373, first column third para et seq.). The medical condition being a tumor/neoplastic disease is disclosed in D1 (abstract). Samples obtained from body fluids are inter alia disclosed in D1 (legend to Figure 1). Therefore, claims 5-8 are not inventive in the light of D1 alone.

5.7 With respect to claim 9:
A method in which the methylation pattern is determined by subjecting the DNA to chemical or enzymatic treatment that converts all unmethylated cytosines to uracil but leaving position 5-methylated cytosines unchanged is not inventive in the light of D1 alone (page 371, second cloumn, second full paragraph).

5.8 With respect to claims 10-11:
The methods of claims 10-11 are considered obvious in the light of the disclosure of D1 (D1, page 372- 373, cf. 'materials and methods' section).

5.9 With respect to claims 12-13:
Claim 12 is considered obvious in the light of a combination of D1 (cf. objections set out supra) and D6, because the latter document discloses a method for characterizing tissues and cell types employing analysis of the methylation pattern (cf. D6, abstract, claims 1, 21, 22) of bisulfite-modified surface-bound (i.e., hybridized) DNA. Thus, the combination of the disclosures of D1 and D6 renders the subject matter of claim 12 obvious to the skilled person. As claim 13 does not add any concept going beyond routine measures, the latter is also considered obvious.

5.10 With respect to claim 14:
Claim 14 considered non-inventive because D1 discloses detection of free floating DNA by amplification methods (D1, abstract).

5.11 With respect to claim 15:
Document D6 discloses a kit comprising s surface to bind free floating DNA, a means for detecting the amount of DNA bound to the surface, reagents to detect the surface-bound DNA, reagents to modify the bound DNA. A means to adjust the temperature in the chamber is not explicitly mentioned rendering claim 15 novel. However, claim 15 is obvious in the light of D6 alone, because the skilled person performing an enzymatic extension or detection reaction would be well aware of the necessity to maintain a temperature at which the enzyme is working properly.

E30109PCT
Epigenomics AG

Claims (amended)

1. A method for detecting the presence or absence of a diseased condition in a tissue, cell type or organ of an individual, comprising the following steps:
   a) obtaining a bodily fluid sample from said individual;
   b) determining the amount or presence of free floating DNA that originates from said tissue, cell type or organ in said sample; and
   c) determining the presence or absence of a diseased condition based on the amount or presence of free floating DNA that originates from said tissue, cell type or organ.

2. A method for detecting the presence or absence of a diseased condition in a tissue, cell type or organ of an individual, comprising the following steps:
   a) obtaining a bodily fluid sample from said individual;
   b) determining the amount of total free floating DNA in said sample;
   c) determining the amount of free floating DNA that originates from said tissue, cell type or organ in said sample; and
   d) determining the presence or absence of a diseased condition based on the total amount of free floating DNA and the fraction of free floating DNA that originates from said tissue, cell type or organ.

3. The method according to claim 1 or 2, characterised in that the sample is conditioned before the amount or presence of free floating DNA is determined.

4. The method according to claim 3, characterised in that the sample is conditioned by means of centrifugation, filtering, heating, cooling, concentration or chemical treatment.

5. The method according to one of the preceding claims, characterised in that the amount or presence of DNA originating from a certain organ or tissue is determined by analysing a DNA methylation pattern that is characteristic for said organ, tissue or cell type.

6. The method according to claim 5, characterised in that said methylation pattern is characteristic for said organ, tissue or cell type and not found in other organs, tissues or cell types involved in the medical condition of interest.

7. The method according to any of the preceding claims, characterised in that the diseased condition is a cell proliferative and/or neoplastic disease.

8. The method according to any of the preceding claims, characterised in that the samples are obtained from bodily fluids like whole blood, blood plasma, blood serum, urine, sputum, ejaculate, semen, tears, sweat, saliva, lymph fluid, bronchial lavage, pleural effusion, peritoneal fluid, meningal fluid, amniotic fluid, glandular fluid, fine needle aspirates, nipple aspirate fluid, spinal fluid, conjunctival fluid, vaginal fluid, duodenal juice, pancreatic juice, bile and cerebrospinal fluid from said individual.

9. The method according to one of the preceding claims, characterised in that the methylation pattern is determined by subjecting the DNA to a chemical or enzymatic treatment that converts all unmethylated cytosines in the DNA into uracil but leaves position 5-methylated cytosines unmodified.

10. A method for detecting the absence or presence of a diseased condition in an organ, cell type or tissue, comprising performing the following steps:
    a) retrieving a bodily fluid sample;
    b) determining the amount or presence of free floating DNA that exhibits a tissue-, organ- or cell type-characteristic DNA methylation pattern;
    c) concluding, whether there is an abnormal level of free floating DNA that originates from said tissue, cell type or organ; and
    d) concluding, whether a diseased condition associated with said tissue, cell type or organ is absent or present.

11. A method for detecting the absence or presence of a diseased condition in a specific organ, cell type or tissue, comprising the following steps:
    a) retrieving a bodily fluid sample;
    b) detecting the amount of total free floating DNA in said sample;
    c) determining the amount of free floating DNA that originates from said specific tissue, cell type or organ by determining free floating DNA that exhibits a tissue-, cell type- or organ-characteristic DNA methylation pattern;
    d) determining the fraction of total free floating DNA that originates from said specific tissue, cell type or organ;
    e) concluding, whether an abnormal level of free floating DNA that originates from said

specific tissue, cell type or organ is present; and

f) concluding, whether a diseased condition associated with said specific tissue, cell type or organ is absent or present.

12. A method for determining the fraction of free floating DNA in a bodily fluid that originates from an organ, cell type or tissue of interest, comprising the following steps:

a) retrieving a bodily fluid sample;

b) conditioning said sample in order to allow a binding of free floating DNA to a surface;

c) binding an essential fraction of said total free floating DNA to said surface;

d) detecting the amount of total free floating DNA by measuring the amount of DNA bound to said surface;

e) subjecting said surface comprising said bound DNA to a chemical and/or enzymatic treatment that converts all unmethylated cytosines in the DNA into uracil but leaves position-5 methylated cytosines unmodified;

f) amplifying the treated DNA;

g) analysing several methylation-specific positions in said treated DNA, and thereby determining the amount of DNA that exhibits a tissue, cell type or organ-characteristic DNA methylation pattern; and

h) determining the fraction of total free floating DNA that originates from said tissue, cell type or organ.

13. The method of claim 12, comprising the following additional step:

i) concluding, whether said DNA originates from said tissue, cell type or organ, if there is an abnormal level of total free floating DNA; and

j) concluding, whether a diseased condition associated with said tissue, cell type or organ is present.

14. The method according to any of the preceding claims, characterised in that the total amount of free floating DNA is measured by intercalating fluorescent dyes or other dyes changing their fluorescence properties when binding to DNA, hybridisation to DNA specific probes such as oligonucleotides or PNA oligomers, real time PCR assays or other real time amplification procedures, UV-Vis absorbance or in general amplification procedures with subsequent determination of the amount of product formed.

15. A kit for determining the total amount of free floating DNA in serum, comprising:

- a surface to bind DNA floating in a sample volume of bodily fluid,
- a means for detecting the amount of DNA bound to this solid surface,
- reagents to chemically or enzymatically modify the DNA bound to the surface,
- a container to host the surface and said reagents, and
- a means to control and adjust the temperature in this chamber.